THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 257, No. 9, Issue of May 10, pp. 5274-5282, 1982
Printed in U.S.A.

On the Chromatin Structure of the Amplified, Transcriptionally Active Gene for Dihydrofolate Reductase in Mouse Cells*

(Received for publication, September 24, 1981, and in revised form, January 4, 1982)

James Barsoum‡, Louis Levinger§, and Alexander Varshavsky¶

From the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The method for two-dimensional hybridization mapping of nucleosomes (Levinger, L., Barsoum, J., and Varshavsky, A. (1981) *J. Mol. Biol.* 146, 287-304) was used to analyze chromatin structure of the gene for dihydrofolate reductase (DHF reductase; 5,6,7,8-tetrahydrofolate:NADP$^+$ oxidoreductase (EC 1.5.1.3)) in L5178Y-R mouse cells. The DHF reductase gene in these cells is amplified about 350-fold as a result of selection for resistance to methotrexate. Dramatic overproduction of DHF reductase mRNA in L5178Y-R cells suggests that most of the DHF reductase genes in these cells are transcribed.

We report that all major mononucleosomal species resolvable by two-dimensional fractionation are detected by both DHF reductase- and satellite DNA-specific hybridization probes. Although the DHF reductase and satellite hybridization patterns differ somewhat from each other and from the total mononucleosomal pattern, their overall similarity is very high. In particular, no large differences in the abundance of mononucleosomes containing high mobility group non-histone proteins (HMG) 14 and 17 are seen between the DHF reductase and satellite chromatin regions under a wide variety of conditions for chromatin isolation, digestion, and fractionation. Possible interpretations of the apparent lack of selectivity of HMG-chromatin interactions in this system are discussed.

We also found that the amplified DHF reductase genes possess a wide range of nucleosomal repeat lengths close to that in the bulk chromatin. In contrast, the range of nucleosomal repeat lengths in the satellite chromatin is much narrower than in both DHF reductase and bulk chromatin.

Most of the chromatin in a eukaryotic cell is organized into nucleosomal fibers (for a review see Kornberg, 1981). Both compositional and conformational heterogeneity along the fibers are observed (for reviews see McGhee and Felsenfeld, 1980; Mathis *et al.*, 1980). Chromatin functions including replication, transcription, and mitotic compaction might be expected to require the non-random distribution of minor chromosomal proteins and/or specific modifications of major proteins, such as histones, at different moments of the cell cycle and in different regions of the genome. In particular, transcriptionally active chromatin regions were shown to differ significantly from the bulk of nontranscribed chromatin in their susceptibility to nucleases, solubility, and other parameters (Weintraub and Groudine, 1976; Garel and Axel, 1976; Levy-Wilson *et al.*, 1979; Bakayev *et al.*, 1979). Since these features of active chromatin are certainly not confined to promoter regions only (Weintraub and Groudine, 1976; Panet and Cedar, 1977), there should exist distinct compositional and/or conformational differences between transcribed and nontranscribed chromatin fibers.

One of the better defined compositional differences between transcribed and nontranscribed chromatin discovered so far is the preferential association of the non-histone proteins, HMG14[1] and HMG17 (Walker *et al.*, 1977; Vidali *et al.*, 1977; Isackson and Reeck, 1981), with the transcribed chromatin isolated from terminally differentiating cells, in particular, from chicken erythroblasts (Weisbrod *et al.*, 1980; Gazit *et al.*, 1980). Purified HMG14 and -17 preferentially bind *in vitro* to globin DNA-containing mononucleosomes from chicken erythroblasts (Weisbrod and Weintraub, 1981; Sandeen *et al.*, 1980; see also Mardian *et al.*, 1980). Structural features of transcribed, globin DNA-containing nucleosomes that determine their preferential binding of HMGs remain unknown, although a correlation exists with undermethylation of transcribed DNA (Mandel and Chambon, 1979; Van der Ploeg and Flavell, 1980; Shen and Maniatis, 1980; Groudine *et al.*, 1981).

We have recently developed a new approach to the analysis of associations of variant nucleosomes with specific DNA sequences (Levinger *et al.*, 1981). The approach consists of two-dimensional fractionation of nucleosomes (low ionic strength electrophoresis of deoxyribonucleoproteins in the first dimension followed by DNA electrophoresis in the second dimension), with subsequent electrophoretic transfer of nucleosomal DNA to DBM paper for hybridization with specific DNA probes. Positions of DNA spots in a two-dimensional DNP-DNA pattern are a function of nucleosome composition and/or conformation in the first (DNP) dimension. Protein composition of separated nucleosomes can be determined by running parallel second dimension protein gels. This method therefore enables one to deduce composition of subsets of

[1] The abbreviations used are: HMG, high mobility group non-histone proteins; DHF reductase, dihydrofolate reductase; DBM paper, diazobenzyloxymethyl paper; PMSF, phenylmethylsulfonyl fluoride; TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; TLCK, N^{α}-*p*-tosyl-L-lysine chloromethyl ketone; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid). Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; SDS, sodium dodecyl sulfate; DNP, deoxyribonucleoproteins; bp, base pairs; kb, kilobase pairs; MN1, core mononucleosome containing 146-bp DNA fragment, core histone octamer but neither histone H1 nor HMG proteins nor ubiquitin-H2A semihistone; MN_{HMG}, a subset of mononucleosomes that contain proteins HMG14 and -17; MN2, a metastable mononucleosomal intermediate containing from ~160- to ~180-bp-long DNA fragment, core histone octamer, and 1 molecule of histone H1.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by a departmental training grant from the National Institutes of Health.

§ Supported by a postdoctoral fellowship from the National Institutes of Health.

¶ Supported by a grant from the National Institutes of Health.

Downloaded from http://www.jbc.org/ at CALIFORNIA INSTITUTE OF TECHNOLOGY on November 25, 2020

nucleosomes containing DNA sequences hybridizing to a specific probe. Application of this approach to the chromatin from HeLa cells (Levinger *et al.*, 1981) has shown that highly repetitive, presumably transcriptionally inactive regions of HeLa chromatin contain significant proportions of the total HMG14 and -17. A similar conclusion was reached by Mathew *et al.* (1981) using a different experimental approach. The presence of HMG proteins in heterochromatic, apparently transcriptionally inactive chromatin regions indicated that specific interactions of HMG proteins with transcribed genes, detected previously by other methods in other systems, are apparently superimposed over a significant background of nonspecific HMG-chromatin interactions.

For further studies we turned to L5178Y-R mouse cells obtained by selection for resistance to high concentrations of methotrexate, a specific inhibitor of the key cellular enzyme, dihydrofolate reductase (Dolnick *et al.*, 1979). The DHF reductase gene dosage in these cells is approximately 350 times higher than in parental, methotrexate-sensitive cells (Schimke *et al.*, 1977; Dolnick *et al.*, 1979; Varshavsky, 1981). Dramatic overproduction of DHF reductase mRNA in L5178Y-R cells (see below) suggests that most of the DHF reductase genes in these cells are transcribed. Although DHF reductase mRNA and enzyme production in mouse cells has been shown to be highest in early S phase (Johnson *et al.*, 1978), recent data by Kellems[2] indicate that this increase is a consequence of post-transcriptional control and that the DHF reductase gene is transcribed at an approximately constant rate throughout the cell cycle.

We analyzed the chromatin structure of the amplified DHF reductase gene using the two-dimensional hybridization mapping approach and report a lack of preferential binding of HMG14 and -17 to the DHF reductase chromatin regions as compared with both satellite-containing chromatin and total chromatin from the L5178Y-R cells. We discuss possible interpretations of these results which are not necessarily incompatible with the concept of preferential HMG binding to certain active genes.

EXPERIMENTAL PROCEDURES

Cell Culture and in Vivo Labeling—Suspension cultures of L5178YR(C3) cells (a gift from Dr. J. Bertino, Yale University Medical School, New Haven, CT) were propagated in plastic tissue culture flasks (Corning) in Fischer's medium for leukemic cells of mice (Gibco) supplemented with penicillin (50 units/ml), streptomycin (50 μg/ml), 10% horse serum (Gibco), and 0.5 mM methotrexate ((+)-amethopterin, Sigma). Monolayer cultures of 3T6 cells (Todaro and Green, 1963) were grown in Eagle's minimal essential medium supplemented with penicillin/streptomycin and 10% calf serum (Gibco).

For DNA labeling of C3 cells, [*Me*-^{3}H]thymidine (New England Nuclear, 20 Ci/mmol) was added to the medium at 2–5 μCi/ml for approximately 20 h. Labeling was performed at an initial cell density of about 8×10^4 cells/ml which allowed exponential cell growth throughout the labeling period. Protein labeling of exponentially growing C3 cells was accomplished by the addition of L-[^{3}H]lysine (New England Nuclear, 60 Ci/mmol) at 8 μCi/ml for approximately 24 h.

Quantitation of DHF Reductase mRNA—Total cytoplasmic RNA was isolated from C3 and 3T6 cells by the method of Berger and Birkenmeier (1979). Poly(A)$^+$ RNA was purified by passing the total cytoplasmic RNA twice through oligo(dT)-cellulose columns (Berger and Cooper, 1975). RNA in separated poly(A)$^-$ and poly(A)$^+$ fractions was precipitated with ethanol, resuspended in 1.5 M glyoxal, 50% dimethyl sulfoxide, 10 mM Na-phosphate (pH 7.0) and incubated for 1 h at 50 °C (McMaster and Carmichael, 1977). After cooling the samples to room temperature, sucrose was added to 5% and the samples (20 μg of poly(A)$^+$ RNA or 200 μg of poly(A)$^-$ RNA per well) were loaded onto a vertical 1.5% agarose gel (0.3 cm thick, 30 cm long) containing 1 mM Na-EDTA, 10 mM Na-phosphate (pH 7.0). Running buffer was the same and electrophoresis was at 3.5 V/cm for 12 h. After electrophoresis, the gel was placed in 50 mM NaOH for 1 h at room temperature, followed by incubation at the same temperature in 2 μg/ml of ethidium bromide, 0.2 M Na-acetate (pH 4.0) for 30 min and then for an additional 15 min at 4 °C. After photographing RNA patterns using 366 nm UV illumination, RNA fragments were transferred from the gel to a DBM paper (Alwine *et al.*, 1977) at 4 °C by blotting (Southern, 1975) using 0.2 M Na-acetate (pH 4.0) as transfer buffer.

Cloned DHF reductase cDNA (pDHFR11, a gift from Dr. R. Schimke) labeled with ^{32}P by nick translation (Rigby *et al.*, 1977) was hybridized to RNA on the DBM paper as described previously (Levinger *et al.*, 1981) and autoradiography was performed at −70 °C using Kodak XAR-5 film and DuPont Lightning Plus intensifying screen.

Quantitation of DHF Reductase Protein—Approximately 3×10^7 of either 3T6 or C3 cells were pelleted and resuspended in 0.25 ml of 0.5% Nonidet P-40, 0.3 M sucrose, 1 mM phenylmethylsulfonyl fluoride (freshly added from 0.5 M stock in absolute ethanol), 3 mM $CaCl_2$, 10 mM Tris-HCl (pH 7.5). The suspension was vortexed to facilitate cell lysis and then centrifuged at 12,000 × *g* for 30 s. The supernatant (cytosol) and pellet (nuclei) were then analyzed by electrophoresis in 18% polyacrylamide-SDS gels (1.5 mm thick, 30 cm long) as described by Thomas and Kornberg (1975). Proteins were stained with Coomassie brilliant blue R-250.

Determination of DHF Reductase Gene Dosage—A modified dot hybridization technique of Kafatos *et al.* (1979) was used as was previously described in detail (Varshavsky, 1981).

Chromatin Preparation and Nuclease Digestion—Several different protocols were used.

1. Labeled C3 cells were centrifuged at 500 × *g* for 5 min at 4 °C, resuspended in 0.14 M NaCl, 5 mM Tris-HCl (pH 7.5), and centrifuged again. The pellet was resuspended in 0.5% Nonidet P-40, 5 mM Na-butyrate, 1 mM PMSF, 5 mM Na-EDTA, 10 mM Na-Hepes (pH 7.5) by gentle strokes in a loosely fitted Dounce homogenizer. After centrifugation at 2,000 × *g* for 5 min, the nuclear pellet was washed once in the lysis buffer and pelleted again. This pellet was then washed twice for a total time of about 1 h in 0.1 mM PMSF, 0.1 mM $CaCl_2$, 1 mM Na-Hepes (pH 7.5) and finally resuspended in the same buffer containing 0.2 mM $CaCl_2$ to approximately 1 mg of DNA per ml. Digestion was carried out by the addition of staphylococcal nuclease (Sigma) to a final concentration of 5 μg/ml followed by incubation at 37 °C. Aliquots were taken at various points from 40 s to 20 min digestion time and added to ⅒ volume of 50 mM Na-EDTA, 25 mM Na-EGTA (pH 7.5). Insoluble material was pelleted at 12,000 × *g* for 5 min. Supernatants were used immediately or frozen at −70 °C in the presence of 10% glycerol. Freezing and thawing did not influence the results obtained.

2. Chromatin was isolated and digested in the presence of divalent cations throughout the procedure. The method is essentially identical with the RSB protocol of Weintraub and Groudine (1976) except that 0.25 M sucrose was added to all buffers to reduce nuclear clumping. Chromatin from digested nuclei was solubilized in 1 mM Na-EDTA, 0.5 mM Na-EGTA, 0.2 mM PMSF, 1 mM Na-Hepes (pH 7.5).

3. Chromatin was isolated and digested as in protocol 1 except that 5,5′-dithiobis-(2-nitrobenzoic acid) (Sigma), a thiol-specific reagent (Wells and Yount, 1980), was present throughout at 0.5 mM during chromatin isolation and at 0.1 mM during nuclease digestion (see Boyce *et al.*, 1982 for the use of DTNB in isolation of SV40 viral nucleoprotein complexes). In addition, all buffers contained protease inhibitors PMSF (0.1 mM), TPCK (50 μM), and TLCK (50 μM).

4. Cells were washed as in protocol 1 and then lysed in 0.5% Nonidet P-40, 0.1 M NaCl, 0.25 M sucrose, 2 mM Na-EDTA, 0.5 mM PMSF, 10 mM Na-Hepes (pH 7.5). The nuclear pellet was washed again in lysis buffer, then twice in 0.25 M sucrose, 0.1 M NaCl, 0.5 mM $CaCl_2$, 0.1 mM PMSF, 10 mM Na-Hepes (pH 7.5). Digestion with staphylococcal nuclease was carried out in this buffer. Digestion was terminated by the addition of EDTA and EGTA to final concentrations of 2 mM and 1 mM, respectively. The suspension was incubated at 4 °C for 30 min with intermittent vortexing and then centrifuged for 8 min at 12,000 × *g* to pellet nonsolubilized chromatin.

Two-dimensional Electrophoretic Analysis of Oligonucleosomes—DNP particles from staphylococcal nuclease-digested C3 chromatin were electrophoresed in the first dimension in a low ionic strength agarose gel followed by second dimension electrophoresis of their DNA fragments in an SDS-agarose gel essentially as described previously for chromatin from rat 14B cells (Levinger *et al.*, 1981). The gel was stained with ethidium bromide followed by hybridization analysis (see below).

[2] R. Kellems, personal communication.

Downloaded from http://www.jbc.org/ at CALIFORNIA INSTITUTE OF TECHNOLOGY on November 25, 2020

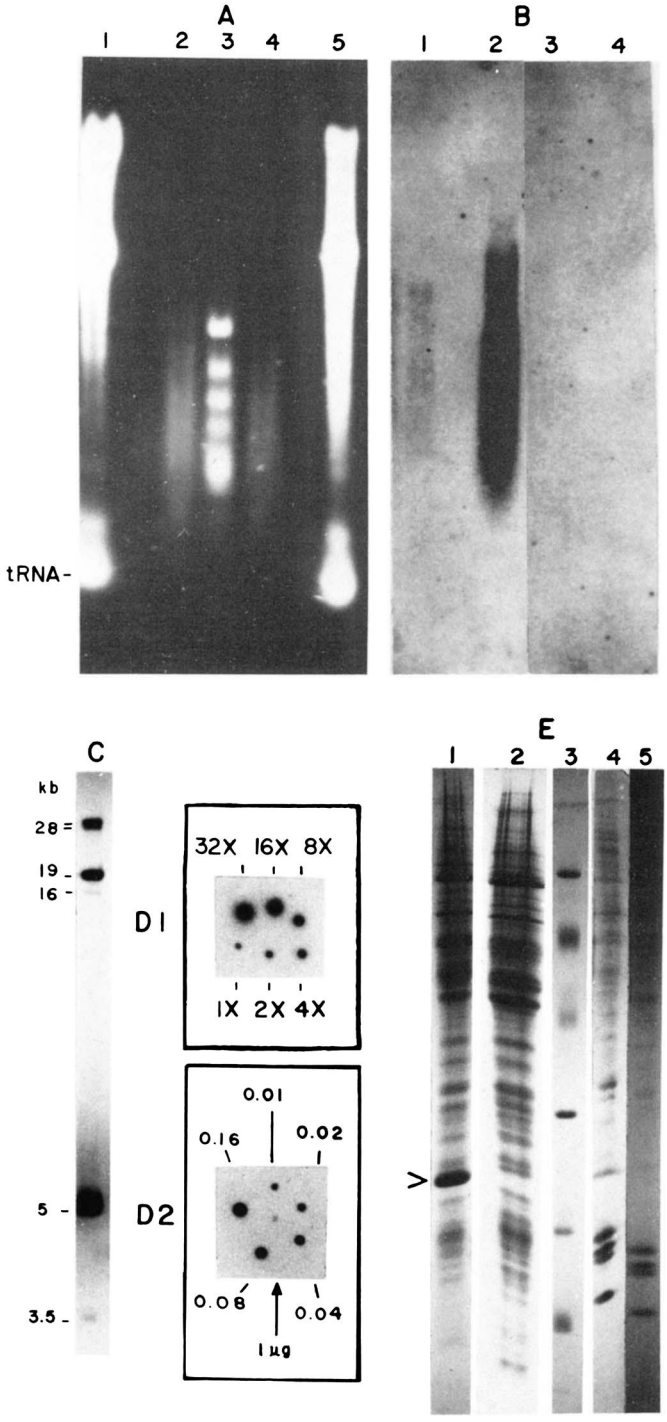

FIG. 1. **Comparison of dihydrofolate reductase gene, RNA, and protein dosages in methotrexate-resistant L5178Y-R(C3) cells and methotrexate-sensitive 3T6 cells.** *A,* total cytoplasmic RNA was extracted from C3 and 3T6 cells and poly(A)$^+$ RNA was purified as described under "Experimental Procedures." Denatured RNA samples were electrophoresed in a 1.5% agarose gel and visualized by staining with ethidium bromide. *Lane 1,* approximately 200 µg of poly(A)$^-$ RNA from C3 cells; *lane 2,* approximately 20 µg of poly(A)$^+$ RNA from C3 cells; *lane 3, Hin*cII-digested, denatured φX174 RF DNA (a marker); *lane 4,* same as in *lane 2* but from 3T6 cells; *lane 5,* same as in *lane 1,* but from 3T6 cells. *B,* fractionated RNA shown in *A* was transferred to DBM paper and hybridized with ^{32}P-labeled pDHFR11. *Lane 1,* poly(A)$^-$ C3 RNA; *lane 2,* poly(A)$^+$ C3 RNA; *lane 3,* poly(A)$^+$ 3T6 RNA; *lane 4,* poly(A)$^-$ 3T6 RNA. *C,* purified high molecular DNA from C3 cells was digested with *Bam* HI, electrophoresed in an 0.8% agarose gel, transferred to a nitrocellulose filter, and hybridized to the [^{32}P]pDHFR11. Lower autoradiographic exposures of the hybridization pattern show that the 28-kb DHF reductase-specific band is a doublet. *D,* relative DHF reductase gene dosage was determined by a dot hybridization technique (Kafatos *et al.*, 1979) as described previously (Varshavsky, 1981) using pDHFR11 as a probe. *D1,* standard ([^{32}P]DNA spotted in a 2-fold dilution series). *D2,* 1 µg of the purified 3T6 DNA was spotted in the center of the nitrocellulose filter and 0.01 to 0.16 µg of the purified C3 DNA was spotted around it. *E,* protein fractions from equal amounts of C3 and 3T6 cells were run in an SDS-polyacrylamide gel and stained with Coomassie. *Lane 1,* C3 cytoplasmic fraction; *lane 2,* 3T6 cytoplasmic fraction; *lane 3,* marker channel; *lane 4,* C3 nuclear fraction; *lane 5,* 3T6 nuclear fraction. *Arrow* to the *left* of *lane 1* indicates the 21.5-kilodalton band of the DHF reductase protein. Marker proteins are: phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (39,000), α-lactalbumin (20,000), and soybean trypsin inhibitor (14,000) (Pharmacia).

Two-dimensional Electrophoretic Analysis of Mononucleosomes—Mononucleosomal DNP particles were resolved in the first dimension by low ionic strength electrophoresis in a 6% polyacrylamide gel (Varshavsky *et al.*, 1977; Levinger and Varshavsky, 1980; Levinger *et al.*, 1981).

Second dimension electrophoresis of the DNA fragments was in a 9% SDS-polyacrylamide gel as described previously (Levinger *et al.*, 1981). The gel was stained with ethidium bromide followed by hybridization analysis (see below).

In experiments in which chromatin was isolated and digested as described in protocol 4 above, solubilized mononucleosomes were fractionated in the first dimension in 5% polyacrylamide gels containing either TBE buffer (89 mM Tris, 89 mM boric acid, 2.5 mM Na-EDTA, pH 8.3) or 0.1 M NaCl, 1 mM Na-EDTA, 0.5 mM Na-EGTA, 10 mM Na-Hepes (pH 7.5).

Second dimension protein runs were carried out in acetic acid-urea-polyacrylamide gels (Panyim and Chalkley, 1969) as described previously (Levinger *et al.*, 1981).

DNA Transfer and Hybridization—DNA was denatured with NaOH and transferred from both one- and two-dimensional agarose gels to nitrocellulose filters by blotting (Southern, 1975). Low molecular weight DNA from two-dimensional polyacrylamide gels was denatured by heating the gel at 100 °C and electrophoretically transferred as described previously (Levinger *et al.*, 1981) to DBM paper (Alwine *et al.*, 1977). Hybridization of transferred DNA with ^{32}P-labeled pDHFR11 (Nunberg *et al.*, 1980) or mouse satellite DNA probes were carried out as described previously for other probes (Levinger *et al.*, 1981). The purified mouse satellite DNA was a gift from Dr. J. Maio (Albert Einstein College of Medicine, New York, NY).

RESULTS

Characterization of Methotrexate-resistant C3 Cells—L5178Y-R (C3) is a murine lymphoblastoid cell line selected for resistance to methotrexate concentrations of up to 1 mM (Dolnick *et al.*, 1979). Methotrexate resistance in these cells is due to a massive amplification of the gene for DHF reductase (Schimke *et al.*, 1977). The amplified DHF reductase genes are located in one or a few contiguous chromosomal regions called "homogeneously staining regions" (Biedler and Spengler, 1976; Dolnick *et al.*, 1979).

We determined the DHF reductase gene dosage in the C3 cells by a modified dot hybridization assay (Kafatos *et al.*, 1979; Thomas, 1980) as described previously (Varshavsky, 1981). As can be seen from Fig. 1, *D1* and *D2*, 0.01 µg of C3 DNA binds a 3 to 4 times greater amount of the DHF reductase hybridization probe than 1 µg of DNA from methotrexate-sensitive 3T6 mouse cells. We conclude that the DHF reductase gene is amplified 300 to 400 times in C3 cells, in good agreement with earlier estimates obtained by C_0t curve analysis (Dolnick *et al.*, 1979). In this case and elsewhere when quantitative estimates were required, we determined relative intensities by taking several different exposures of each autoradiogram and determining the difference in the time of exposure needed to produce an equal intensity in the species in question. The hybridization probe used in these and subsequent experiments, pDHFR11, hybridizes to several dis-

Downloaded from http://www.jbc.org/ at CALIFORNIA INSTITUTE OF TECHNOLOGY on November 25, 2020

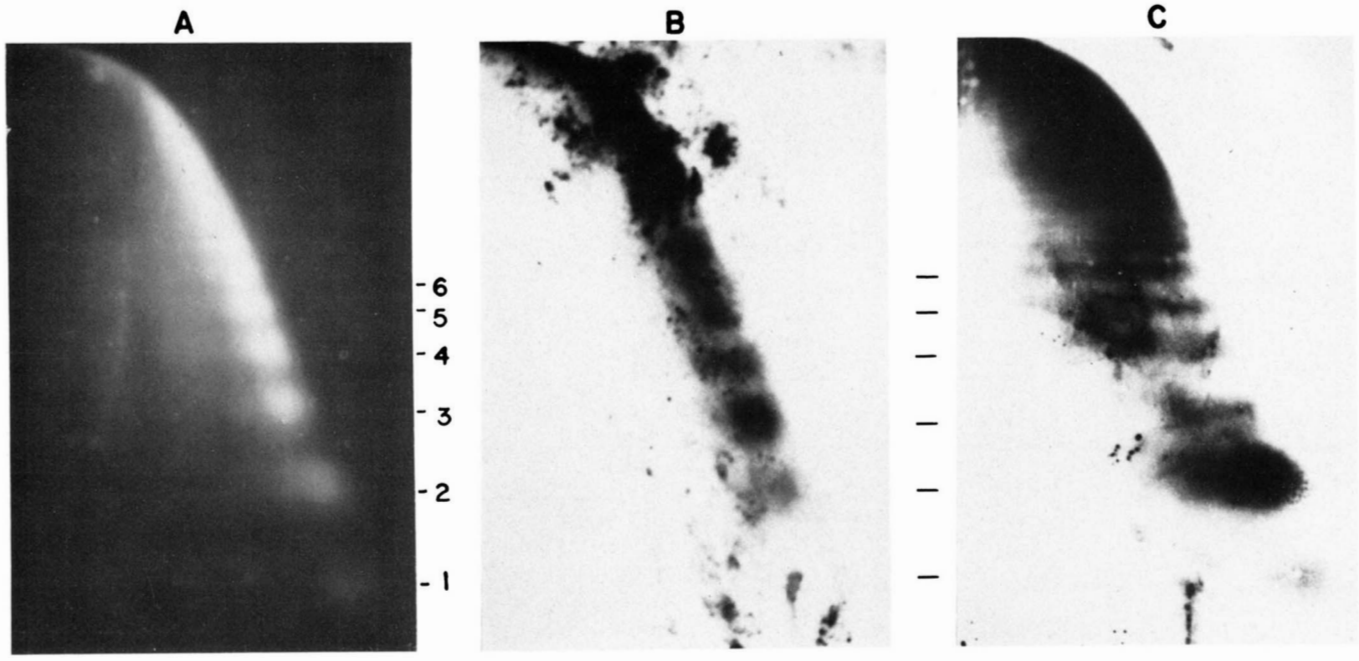

FIG. 2. **Two-dimensional hybridization mapping of C3 oligonucleosomes.** Staphylococcal nuclease-digested C3 chromatin (prepared according to protocol 1 under "Experimental Procedures") was fractionated in the first dimension on a low ionic strength 0.8% agarose gel followed by a second dimension DNA run in a 2.2% agarose-SDS gel. *A*, the total DNP → DNA pattern (ethidium-stained). *B*, the DNA in *A* was transferred to DBM paper and hybridized to the [^{32}P]pDHFR11 probe. *C*, the same DBM paper was rehybridized (see "Experimental Procedures") to the ^{32}P-labeled mouse satellite DNA probe.

crete DNA species in the fractionated *Bam* HI digest of the purified C3 DNA (Fig. 1*C*), in agreement with the results of Nunberg *et al.* (1980).

We also compared titers of the DHF reductase mRNA in C3 and 3T6 cells (Fig. 1, *A* and *B*). Total cytoplasmic RNAs from C3 and 3T6 cells were isolated and fractionated into poly(A)$^+$ and poly(A)$^-$ fractions, then electrophoresed in an agarose gel (Fig. 1*A*), transferred to DBM paper, and hybridized to the cloned [^{32}P]DHF reductase cDNA probe (Fig. 1*B*).

Fig. 1, *A* and *B* shows that for a constant amount of the total poly(A)$^+$ RNA there is an immense excess of the DHF reductase mRNA from C3 cells over that from 3T6 cells. The difference is at least 250-fold and may be even higher, suggesting that a considerable proportion, if not all, of the amplified DHF reductase genes are transcriptionally active. Not distinguished in *lane 2* of Fig. 1*B*, but clearly seen upon lower autoradiographic exposure (not shown), are four DHF reductase poly(A)$^+$ RNA species of about 1600, 1200, 1000, and 750 bases long, in agreement with the data of Setzer *et al.* (1980).

Polyacrylamide-SDS gel analysis of cytoplasmic and nuclear protein fractions from C3 and 3T6 cells (Fig. 1*E*) confirms a great overproduction of the DHF reductase protein in C3 cells (Fig. 1*E1*) and its cytoplasmic localization, in agreement with earlier findings (Schimke *et al.*, 1977; Dolnick *et al.*, 1979).

The Amplified DHF Reductase Genes Possess a Wide Range of Nucleosomal Repeat Lengths—C3 chromatin was digested with staphylococcal nuclease; solubilized oligonucleosomes were electrophoresed in the first (DNP) dimension in an 0.8% low ionic strength agarose gel followed by second dimension DNA electrophoresis (Fig. 2). The gel was stained with ethidium bromide (Fig. 2*A*), and the DNA was transferred to DBM paper for hybridization, at first with the DHF reductase (Fig. 2*B*) and then with the satellite DNA probe (Fig. 2*C*; see "Experimental Procedures").

The total (ethidium-stained) two-dimensional oligonucleosomal DNP-DNA pattern is virtually indistinguishable from the DHF reductase-specific hybridization pattern (compare *A* and *B* of Fig. 2). Thus, the range of nucleosomal DNA repeat lengths in the bulk chromatin is apparently close to if not identical with the range observed in the DHF reductase chromosomal domain. This finding does not by itself eliminate the possibility that nucleosomes are nonrandomly positioned along the ~32-kb-long DHF reductase gene; however, any DHF reductase nucleosome arrangement with a *unique* repeat length is not consistent with these data (Fig. 2*B*).

A very different result was obtained when the mouse satellite DNA probe was hybridized to the same total DNA pattern (Fig. 2*C*). Satellite oligonucleosomal DNA spots are more elongated in the first (DNP) dimension and much thinner in the second (DNA) dimension, strongly suggesting that the range of nucleosomal DNA repeat lengths in the satellite chromatin is much narrower than in either DHF reductase or bulk chromatin (Fig. 2*C*; *cf.* Fig. 2, *A* and *B*). This same result was obtained previously with rat satellite chromatin (Levinger *et al.*, 1981) which has a DNA sequence repeat length of 370 bp (the predominating DNA repeat length in mouse satellite is 240 bp (Reiss and Biro, 1978)). Another interesting feature of the satellite hybridization pattern (Fig. 2*C*) is a very high relative intensity of the dinucleosomal spot; note also that the range of DNA sizes within this spot is far greater than for the rest of satellite-specific oligonucleosomal DNA spots (Fig. 2*C*; *cf.* Fig. 2, *A* and *B*). The structural meaning of these features of the satellite-specific hybridization pattern remains unknown.

Protein Components of Electrophoretically Resolved Mononucleosomes from C3 Cells—Mononucleosomes labeled *in vivo* with [^{3}H]lysine were fractionated in the first (DNP) dimension in a low ionic strength polyacrylamide gel (Fig. 3*B*) followed by second dimension acetic acid-urea-polyacrylamide gel electrophoresis to resolve protein components of the separated mononucleosomal particles (Fig. 3*A* and *inset*). One feature of the protein pattern (Fig. 3*A*) is a virtually complete absence of mononucleosomes containing ubiquitin-H2A semihistone (uH2A; previously called A24 protein), a specific covalent conjugate of histone H2A and a small protein, ubi-

Downloaded from http://www.jbc.org/ at CALIFORNIA INSTITUTE OF TECHNOLOGY on November 25, 2020

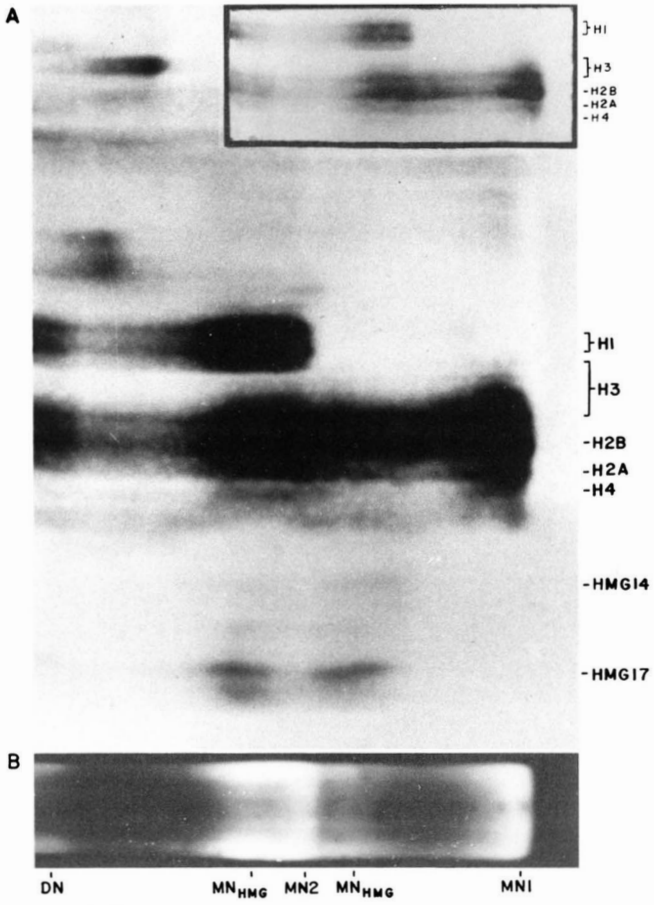

FIG. 3. **Protein components of electrophoretically separated C3 nucleosomes.** L-[^{3}H]Lysine-labeled C3 chromatin was prepared and digested according to protocol 1 under "Experimental Procedures." Second dimension protein analysis of the mononucleosomes fractionated in the first dimension (ethidium-stained first dimension gel is shown in *B*) was carried out in acetic acid-urea-polyacrylamide gel (see "Experimental Procedures"). The *inset* in the *upper right corner* shows a lower fluorographic exposure of H1 and core histone region. Positions of HMG14 and -17 are indicated on the *right*. Designations of DNP particles in *B* are as in Fig. 4.

quitin (Goldknopf *et al.*, 1977; Schlesinger *et al.*, 1975; Wu *et al.*, 1981). The uH2A-containing core (MN1) mononucleosomes migrate slightly slower than uH2A-less MN1 mononucleosomes in the first (DNP) dimension; their characteristic "signature" in the second (protein) dimension is the presence of the uH2A spot, migrating slightly slower than histone H1 (Levinger and Varshavsky, 1980; Levinger *et al.*, 1981). Only extreme fluorographic overexposures of the pattern in Fig. 3*A* revealed the presence of trace amounts of uH2A-containing core mononucleosomes in nuclease digests of C3 chromatin (data not shown). This is in contrast to considerable amounts of uH2A-containing nucleosomes in chromatin preparations from other cultured cells, such as HeLa (Goldknopf *et al.*, 1977; Levinger and Varshavsky, 1980), mouse L1210 cells (Wu *et al.*, 1981), and *Drosophila* (Levinger and Varshavsky, 1982). The relative amount of uH2A in C3 chromatin preparations was not increased even when chromatin isolation was carried out in the presence of proteinase inhibitors PMSF (0.5 mM), TPCK (50 μM), and TLCK (50 μM) and an —SH-specific reagent 5,5′-dithiobis-(2-nitrobenzoic acid) (0.5 mM) (see "Experimental Procedures"; data not shown). It remains to be seen whether the virtual absence of uH2A in chromatin preparations from C3 cells reflects the *in vivo* situation, or whether uH2A is lost during chromatin isolation and nuclease digestion as a result of activation of a uH2A-specific protease *in vitro* (see "Discussion"). Such an activity, which specifically splits uH2A into its components, histone H2A and ubiquitin, was detected recently in liver nuclei from thioacetamide-treated rats (Andersen *et al.*, 1981).

With the exception of the absence of uH2A spots, the overall two-dimensional DNP protein pattern (Fig. 3*A*) is quite similar to the one observed with HeLa mononucleosomes (Levinger *et al.*, 1981). In particular, relative mobilities of mononucleosomes containing proteins HMG14 and HMG17 (these mononucleosomes migrate both faster and slower than the H1-containing mononucleosomes) are essentially the same as those for HeLa HMG-containing mononucleosomes (Fig. 3*A*; *cf.* Levinger *et al.*, 1981). Besides HMG14 and -17, a new HMG-like protein species is detected in HMG-containing mononucleosomes from C3 chromatin. This protein runs slightly ahead of HMG17 in the acetic acid-urea gel and resides in the same area of the two-dimensional DNP → protein patterns as the other two HMGs. Although this rapidly migrating HMG-like protein may be a partial degradation product of either HMG14 or -17, a recent report by Goodwin *et al.* (1981) in which a new HMG protein with similar electrophoretic properties has been identified in calf thymus mononucleosomes, makes it plausible that we are observing the C3 counterpart of the calf HMG protein. Additional minor HMG-like protein bands are present in Fig. 3*A*. Their distribution in the first (DNP) dimension is similar to that for HMG14 and -17 (data not shown). These proteins may be analogous to minor HMG-like proteins observed previously in HeLa mononucleosomes (Levinger *et al.*, 1981).

Two-dimensional Hybridization Mapping of DHF Reductase and Satellite Mononucleosomes—[^{3}H]Thymidine-labeled C3 chromatin was prepared under low ionic strength conditions and digested with staphylococcal nuclease, and the mononucleosomes obtained were electrophoresed in the first (DNP) dimension in a low ionic strength polyacrylamide gel (Fig. 4, *A1* and *B1*, an early and a late digest, respectively). The second dimension DNA run was then carried out (Fig. 4, *A2* and *B2*) and the DNA was denatured *in situ* and electrophoretically transferred to DBM paper (see "Experimental Procedures"). Hybridization of the total two-dimensional DNP-DNA patterns to the DHF reductase probe (Fig. 4, *A3* and *B3*) and thereafter to the satellite DNA probe (Fig. 4, *A4* and *B4*) revealed a striking similarity of the total pattern to both of the hybridization patterns. To visualize the total transferred DNA pattern, the DBM paper was impregnated with 2,5-diphenyloxazole after first removing all hybridized ^{32}P counts as described previously (Levinger *et al.*, 1981; see also "Experimental Procedures"). It should be noted that the presence of [^{3}H]DNA on the DBM paper under the conditions used did not make any contribution to the autoradiographic image of the hybridized [^{32}P]DNA, as was verified by appropriate controls (Levinger *et al.*, 1981). The patterns of total [^{3}H]DNA on DBM paper (Fig. 4, *A5* and *B5*) are also very similar to the hybridization patterns (Fig. 4, *A3*, *A4*, *B3*, and *B4*), although a few minor differences were apparent. For example, in the late digest, the "whiskers" under the MN1 spot (see Levinger and Varshavsky, 1980, for a more detailed description of whiskers) are present to different extents in the total, satellite, and DHF reductase-specific patterns (Fig. 4*B*). The significance, if any, of these minor differences is not known.

The overall similarity of the total and DHF reductase mononucleosomal patterns includes the region of HMG-containing mononucleosomes (Fig. 4). Although most, if not all, of the amplified DHF reductase genes are apparently transcriptionally active in C3 cells (see "Discussion"), and although HMG proteins 14 and 17 are known to interact pref-

Downloaded from http://www.jbc.org/ at CALIFORNIA INSTITUTE OF TECHNOLOGY on November 25, 2020

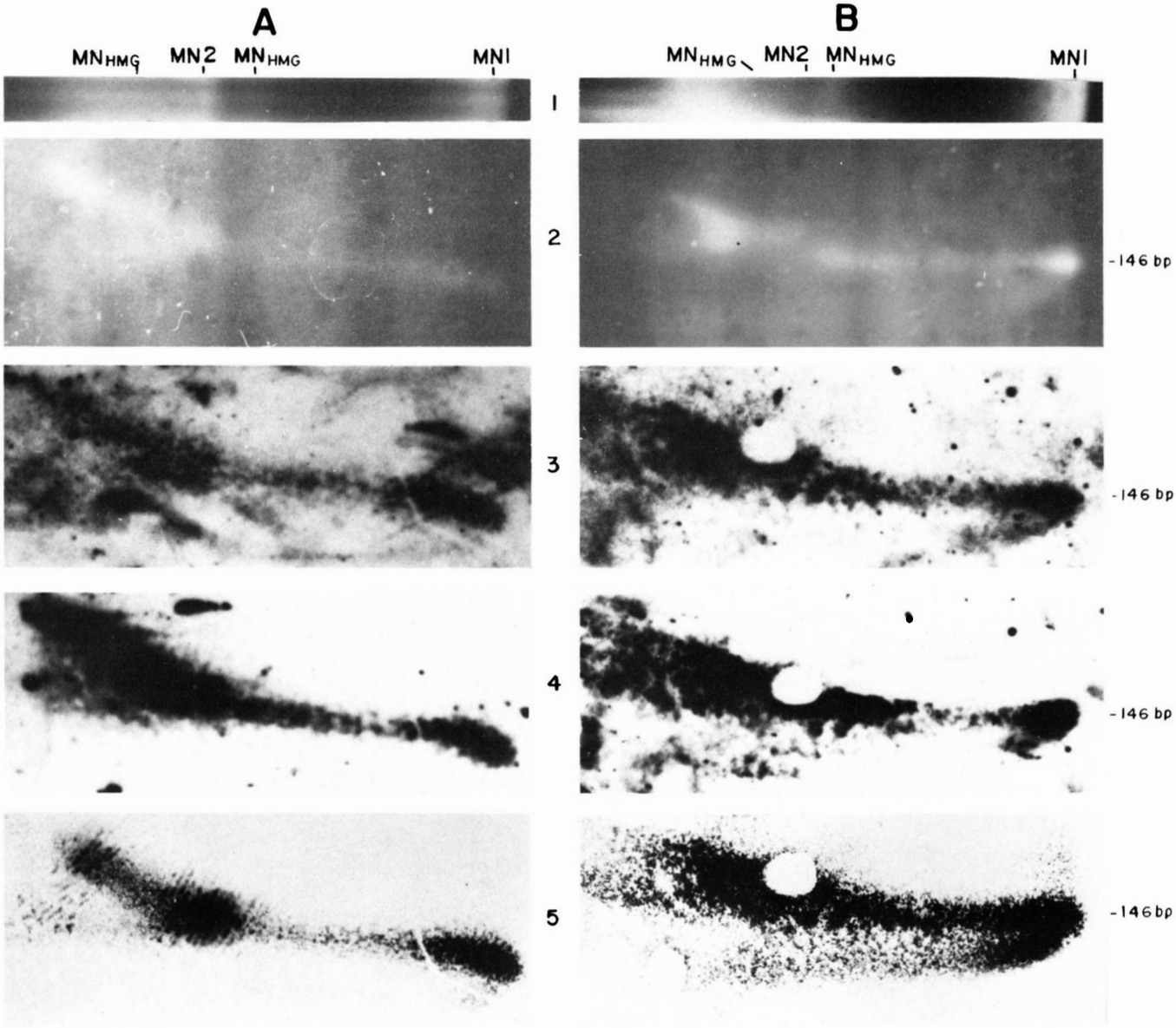

FIG. 4. **Two-dimensional hybridization mapping of DHF reductase and satellite mononucleosomes from C3 cells.** [^{3}H] Thymidine-labeled C3 chromatin prepared and digested with staphylococcal nuclease according to protocol 1 under "Experimental Procedures," was fractionated in the first dimension low ionic strength 6% polyacrylamide gel, followed by a second dimension DNA run in a 9% SDS-polyacrylamide gel. *A* and *B* correspond to 3-min and 20-min nuclease digests, respectively. *1,* ethidium-stained first dimension DNP patterns. *2,* ethidium-stained second dimension DNA patterns. *3,* DNA was transferred electrophoretically to DBM paper and hybridized to the [^{32}P]pDHFR11 probe. *4,* the same DBM papers were rehybridized (see "Experimental Procedures") with the ^{32}P-labeled satellite DNA probe. *5,* the same DBM papers were treated to remove all hybridized ^{32}P counts (see "Experimental Procedures" and Levinger *et al.*, 1981) and fluorographed in the presence of 2,5-diphenyloxazole to visualize the patterns of transferred ^{3}H-labeled total DNA. Designations: *MN1,* core mononucleosome containing 146-bp DNA fragment, core histone octamer but neither histone H1 nor HMG proteins, nor ubiquitin-H2A semihistone. *MN$_{HMG}$,* a subset of mononucleosomes that contain proteins HMG14 and -17. *MN2,* a metastable mononucleosomal intermediate containing from ~160- to ~180-bp-long DNA fragment, core histone octamer, and 1 molecule of histone H1.

erentially with nucleosomes containing transcribed globin DNA sequences from chicken erythroid cells (Albanese and Weintraub, 1980; Weisbrod and Weintraub, 1981; Sandeen *et al.*, 1980), we do not find a preferential association of HMG14 and -17 with DHF reductase-specific nucleosomes from C3 cells (Figs. 4 and 5).

HMG-containing mononucleosomes (MN$_{HMG}$) migrate in two groups, one just ahead of MN2 and the other just behind it (Fig. 3), similar to HeLa HMG-mononucleosomes (Levinger *et al.*, 1981). The MN$_{HMG}$'s which migrate ahead of MN2 lack H1 histone and contain 1 molecule of either HMG14 or -17 per particle (Albright *et al.*, 1980; Sandeen *et al.*, 1980; Mardian *et al.*, 1980). More than 50% of the total DNA in the corresponding area of the first (DNP) dimension pattern corresponds to MN$_{HMG}$ DNA as determined by comparison of relative intensities of [^{3}H]lysine-labeled or Coomassie-stained HMG and histone H4 bands in different DNP protein patterns (Fig. 3 and data not shown; see Levinger *et al.*, 1981, for a detailed description of the procedure as applied to HeLa mononucleosomes). Composition of MN$_{HMG}$ mononucleosomes which migrate behind MN2 in the first (DNP) dimension (Fig. 3) is mixed, consisting of MN$_{2HMG}$, MN$_{H1,HMG}$, and MN$_{2HMG,H1}$ (Albright *et al.*, 1980). Both of the MN$_{HMG}$ DNA regions, which flank the MN2 DNA spot, are represented equally in the total, DHF reductase-specific, and satellite-specific patterns with relation to "non-HMG" DNA markers

Downloaded from http://www.jbc.org/ at CALIFORNIA INSTITUTE OF TECHNOLOGY on November 25, 2020

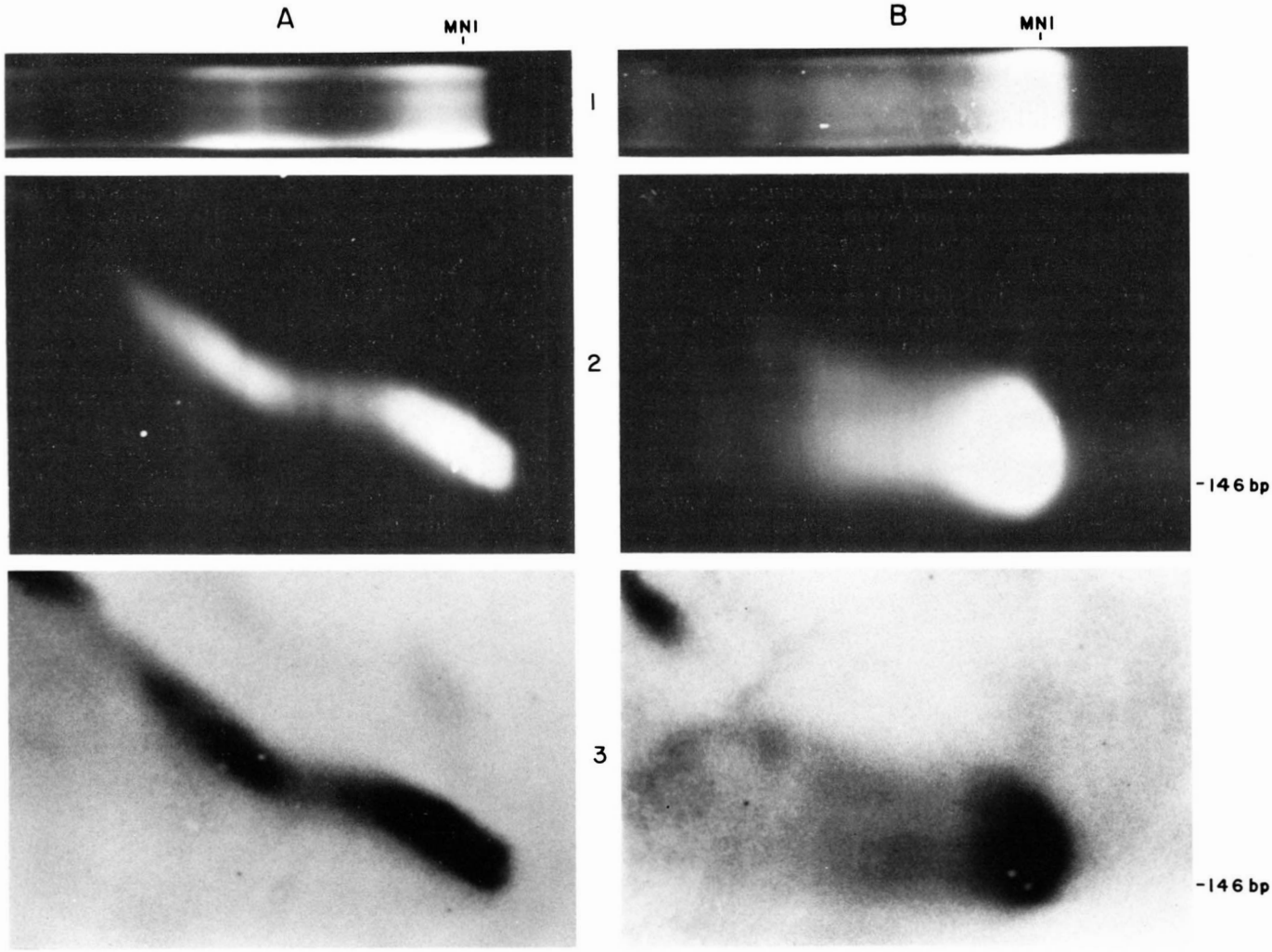

FIG. 5. **Two-dimensional hybridization mapping of DHF reductase mononucleosomes produced and fractionated under high salt conditions.** C3 chromatin was prepared and digested according to protocol 4 under "Experimental Procedures." Mononucleosomes from the same intermediate level digest were fractionated in the first dimension in 5% polyacrylamide gels with either TBE buffer (*A*) or 0.1 M NaCl-containing buffer (*B*) (see "Experimental Procedures" for details). *1*, ethidium-stained first dimension DNP patterns. *2*, ethidium-stained second dimension DNA patterns. *3*, DNA was transferred electrophoretically to DBM paper and hybridized to the [^{32}P]pDHFR11 probe (see "Experimental Procedures").

MN1 and MN2 (Figs. 4 and 5).

To check whether this finding depended on particular conditions of chromatin isolation, digestion, and fractionation, we used different protocols for all three (see "Experimental Procedures"). The procedures included low ionic strength conditions throughout (Fig. 4), similar protocols but in the presence of divalent cations (data not shown), use of several different proteinase inhibitors and of an —SH-specific reagent, DTNB (see "Experimental Procedures"; data not shown), and lastly, more "physiological" concentrations of monovalent cations throughout chromatin isolation, digestion, and fractionation (Fig. 5). The latter methods (see "Experimental Procedures") were used following the results of Sandeen *et al.* (1980), who found that HMG-nucleosome reconstitution and electrophoresis at higher ionic strengths promotes cooperativity of HMG binding, favoring the MN_{2HMG} product. In all cases, a great similarity between the total and DHF reductase-specific hybridization patterns was observed (Figs. 4 and 5), including the relative proportions of HMG-mononucleosomal DNA in the DHF reductase-specific *versus* bulk chromatin regions.

The data of Fig. 5 illustrate a strong dependence of the mononucleosomal patterns on the ionic strength of the first (DNP) dimension electrophoretic system. In our 0.1 M NaCl-based mononucleosome preparation, no well defined, discrete MN2 particles are seen in either the TBE or 0.1 M NaCl gel system (Fig. 5). However, a large proportion of slowly migrating material containing longer than 146-bp DNA fragments, is seen when a relatively low ionic strength first dimension TBE electrophoretic buffer is used (conductivity of TBE is ~⅛ that of 0.1 M NaCl) (Fig. 5*A*). Since HMG-less, H1-containing MN2 particles are insoluble in 0.1 M NaCl (Albright *et al.*, 1980; McGhee and Felsenfeld, 1980), the slowly migrating material in Fig. 5*A* probably consists of $MN_{H1,HMG}$ and/or of MN_{2HMG}. In a 0.1 M NaCl-containing gel, these particles almost co-migrate with MN1, apparently as a result of losing their H1 and/or HMG components during the electrophoresis. The analogous phenomenon of electrophoresis-induced stripping of H1 histone from MN2 mononucleosomes at ionic strengths exceeding ~0.03 was observed by Albanese and Weintraub (1980).

As already mentioned in the preceeding section, isolated C3 chromatin and mononucleosomes were virtually completely devoid of uH2A semihistone, in contrast to the situation with many other cell lines from different species. Our recent studies with *Drosophila* chromatin strongly implicated uH2A as a specific component of transcribed chromosomal fibers (Levinger and Varshavsky, 1982). Since it is still possible that uH2A is present in C3 cells *in vivo* but is cleaved during

Downloaded from http://www.jbc.org/ at CALIFORNIA INSTITUTE OF TECHNOLOGY on November 25, 2020

chromatin isolation *in vitro*, further studies on C3 mononucleosomes would have to rely on some method for inhibiting uH2A-specific isopeptidase activity.

DISCUSSION

Absence of Preferential Binding of HMG14 and -17 to DHF Reductase Genes in Isolated C3 Chromatin: Possible Interpretations—The major result of this work is the conclusion that although the amplified DHF reductase genes are apparently transcribed in C3 cells, there is no preferential association of HMGs with the DHF reductase mononucleosomes in chromatin isolated from unsynchronized C3 cells. Minor differences in HMG content might be missed by our procedure, but it is clear that there are no large differences in HMG content between transcribed DHF reductase and nontranscribed satellite DNA sequences in isolated mononucleosomes (see "Results"). Preferential association of HMGs with DHF reductase mononucleosomes was expected by analogy with results from other laboratories obtained with a very different system, chicken erythroid cells (Weisbrod *et al.*, 1980; Sandeen *et al.*, 1980). According to those data, HMG14 and -17 bind preferentially to globin DNA-containing nucleosomes isolated from terminally differentiating chicken erythroblasts. Our results clearly do not contradict, at least at the present level of understanding, the concept of preferential HMG binding to some active genes; several different interpretations are formally compatible with our data. Below we enumerate and briefly discuss some of these interpretations.

1. The assumption that a considerable proportion of the amplified DHF reductase genes are active in C3 cells may be invalid; if only a small fraction of about 350 DHF reductase gene copies per C3 cell is transcriptionally active, the preferential association of HMGs with these few genes might not be seen over a background of nonspecific HMG interactions with the bulk of inactive DHF reductase genes.

Since no *direct* estimate of the proportion of transcribed DHF reductase genes in an unsynchronized population of C3 cells is available, this interpretation cannot be refuted at the present time. It is unlikely, however, that only a few out of approximately 350 DHF reductase genes are actually transcribed, since first, the degree of DHF reductase gene amplification is a strong function of the selecting methotrexate concentration (Schimke *et al.*, 1977; Varshavsky, 1981), suggesting that all or almost all amplified DHF reductase genes participate in the production of the DHF reductase mRNA. Second, it is unlikely that at least a 250-fold overproduction of the DHF reductase mRNA by C3 cells (see "Results") is due to a very high activity of a small fraction of the amplified DHF reductase genes, in the absence of any activity of the rest of them.

2. It is possible that HMGs do interact preferentially with the DHF reductase genes *in vivo* but redistribute between different nucleosomes during chromatin isolation, digestion, and/or fractionation, so that the *in vitro* HMG distribution is no longer DHF reductase-specific.

Although this interpretation cannot be refuted at the present time, and moreover, nucleosome-bound HMG proteins are known to redistribute *in vitro* under certain solvent conditions (Albright *et al.*, 1980), we showed that a wide variety of chromatin isolation, digestion, and fractionation protocols (see "Experimental Procedures" and "Results") all produce the same apparently nonspecific *in vitro* HMG distribution with regard to the DHF reductase genes. Some of these protocols were identical with those used with the globin gene/chicken erythroid cell system in which preferential HMG binding to nucleosomes containing globin-specific DNA sequences was convincingly demonstrated (Weisbrod and Weintraub, 1981; Sandeen *et al.*, 1980).

3. Current estimates of the level of transcriptional activity of the "housekeeping" DHF reductase gene in noncycling, methotrexate-sensitive cells place it at 10- to 100-fold lower than the level of activity of differentiation-specific "luxury" genes such as those for globin and ovalbumin in terminally differentiating chicken erythroid and oviduct cells (Mathis *et al.*, 1980). It now appears that the DHF reductase gene is transcribed at an approximately constant rate throughout the cell cycle.[2] We have previously suggested that maintenance of preferential associations of HMGs with a transcribed chromatin fiber may be dependent upon frequent passages of transcribing RNA polymerases along the fiber (Levinger *et al.*, 1981). In other words, it is possible that the linear density of HMG binding to nucleosomes along a given chromatin fiber *in vivo* is a function of the frequency of transcription of that fiber. If so, highly active genes, such as the globin gene in erythroid cells, would be expected to have more HMGs bound per nucleosome than the relatively slowly transcribed genes, such as DHF reductase.

Ubiquitin-H2A Semihistone Is Virtually Absent from Isolated C3 Chromatin—One unusual feature of C3 chromatin and mononucleosome preparations was the almost complete absence of uH2A, a specific covalent conjugate of histone H2A with a small protein, ubiquitin (see Fig. 4). uH2A semihistone substitutes for about 5 to 15% of nucleosomal histone H2A in chromatin from a wide variety of animal tissues and established cell lines, from man to *Drosophila* (Goldknopf *et al.*, 1977; Kleinschmidt and Martinson, 1981; Levinger and Varshavsky, 1982). The unique structure of uH2A (Goldknopf *et al.*, 1977), its unusual metabolic properties (Wu *et al.*, 1981), and universal occurrence coupled with the recently discovered participation of free ubiquitin in the functioning of the cytoplasmic ATP-dependent proteolytic system (Hershko *et al.*, 1980; Wilkinson *et al.*, 1980) all suggest that uH2A serves some important chromosomal function.

Application of the technique for two-dimensional hybridization mapping of nucleosomes to *Drosophila* chromatin has led us recently to the discovery that uH2A-containing *Drosophila* mononucleosomes are derived specifically from transcribed chromosomal fibers (Levinger and Varshavsky, 1982).

Therefore, it would be important to know whether the virtual absence of uH2A from isolated C3 chromatin (see "Results") is due to its artifactual degradation by a uH2A-specific isopeptidase (Andersen *et al.*, 1981) during chromatin and mononucleosome preparation *in vitro* or whether the lack of uH2A in cells with strongly amplified specific genes may have some physiological significance.

Acknowledgments—We are greatly indebted to Joseph Maio for the purified satellite DNA, to Robert Schimke and Randal Kaufman for the DHF reductase cDNA clone, to Joseph Bertino and Barbara Morrison for L5178Y-R(C3) cells, and to Vladimir Volloch for advice on the DHF reductase mRNA isolation.

REFERENCES

Albanese, I., and Weintraub, H. (1980) *Nucleic Acids Res.* **8,** 2787–2805

Albright, S. C., Wiseman, J. M., Lange, R. A., and Garrard, W. T. (1980) *J. Biol. Chem.* **255,** 3673–3684

Alwine, J. C., Kemp, D. J., and Stark, G. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 5350–5354

Andersen, M. W., Ballal, N. R., Goldknopf, I. L., and Busch, H. (1981) *Biochemistry* **20,** 1104–1110

Bakayev, V. V., Schmatchenko, V. V., and Georgiev, G. P. (1979) *Nucleic Acids Res.* **7,** 1525–1540

Berger, S. L., and Birkenmeier, C. S. (1979) *Biochemistry* **18,** 5143–5149

Downloaded from http://www.jbc.org/ at CALIFORNIA INSTITUTE OF TECHNOLOGY on November 25, 2020

Berger, S. L., and Cooper, H. L. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72,** 3873–3877
Biedler, J. L., and Spengler, B. A. (1976) *Science* **191,** 185–189
Boyce, F. M., Sundin, O., Barsoum, J., and Varshavsky, A. (1982) *J. Virol.*, in press
Dolnick, R. J., Berenson, R. J., Bertino, J. R., Kaufman, R. J., Nunberg, J. H., and Schimke, R. T. (1979) *J. Cell Biol.* **83,** 394–402
Garel, A., and Axel, R. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73,** 3966–3970
Gazit, B., Panet, A., and Cedar, H. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 630–634
Goldknopf, I. A., French, M. F., Musso, R., and Busch, H. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 5492–5495
Goodwin, G. H., Wright, C. A., and Johns, E. W. (1981) *Nucleic Acids Res.* **9,** 2761–2775
Groudine, M., Eisenman, R., and Weintraub, H. (1981) *Nature* **292,** 311–317
Hershko, A., Ciechanover, A., Heller, H., Haas, A. L., and Rose, I. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 1783–1786
Isackson, P. J., and Reeck, G. R. (1981) *Nucleic Acids Res.* **9,** 3779–3791
Johnson, L. F., Fuhrman, C. L., and Wiedemann, L. M. (1978) *J. Cell Physiol.* **97,** 397–406
Kafatos, F. C., Jones, C. W., and Efstratiadis, A. (1979) *Nucleic Acids Res.* **7,** 1541–1552
Kleinschmidt, A. M., and Martinson, H. G. (1981) *Nucleic Acids Res.* **9,** 2423–2454
Kornberg, R. D. (1981) *Nature* **222,** 579–580
Levinger, L., and Varshavsky, A. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 3244–3248
Levinger, L., and Varshavsky, A. (1982) *Cell* **28,** 375–385
Levinger, L., Barsoum., J., and Varshavsky. A. (1981) *J. Mol. Biol.* **146,** 287–304
Levy, W. B., Connor, W., and Dixon, G. H. (1979) *J. Biol. Chem.* **254,** 609–620
Mandel, J. L., and Chambon, P. (1979) *Nucleic Acids Res.* **7,** 2081–2090
Mardian, J. K. W., Paton, A. E., Bunick, G. J., and Olins, D. E. (1980) *Science* **209,** 1534–1536
Mathis, D., Oudet, P., and Chambon, P. (1980) *Progr. Nucl. Res. Mol. Biol.* **24,** 1–55
Mathew, C. G. P., Goodwin, G. H., Igo-Kemenes, T., and Johns, E. W. (1981) *FEBS Lett.* **125,** 25–29
McGhee, J. O., and Felsenfeld, G. (1980) *Annu. Rev. Biochem.* **49,** 1115–1156
McMaster, G. K., and Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 4835–4838
Nunberg, J. H., Kaufman, R. J., Chang, A. C. Y., Cohen, S. N., and Schimke, R. T. (1980) *Cell* **19,** 355–364
Panet, A., and Cedar, H. (1977) *Cell* **11,** 933–940
Panyim, S., and Chalkley, R. (1969) *Arch. Biochem. Biophys.* **130,** 337–346
Reis, R. J. Shmookler, and Biro, P. A. (1978) *J. Mol. Biol.* **121,** 357–374
Rigby, P. W. J., Dieckmann, M., Rhodes, C., and Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251
Sandeen, G., Wood, W. I., and Felsenfeld, G. (1980) *Nucleic Acids Res.* **8,** 3757–3778
Schimke, R. T., Alt, F. W., Kellems, R. E., Kaufman, R. J., and Bertino, J. R. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 649–657
Schlesinger, D. H., Goldstein, G., and Niall, H. D. (1975) *Biochemistry* **14,** 2214–2218
Setzer, D. R., McGrogan, M., Nunberg, J. H., and Schimke, R. T. (1980) *Cell* **22,** 361–370
Shen, C. K. J., and Maniatis, T. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 6634–6638
Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517
Thomas, J., and Kornberg, R. O. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72,** 2626–2630
Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 5201–5205
Todaro, G. J., and Green, H. (1963) *J. Cell Biol.* **17,** 299–313
van der Ploeg, L. H. T., and Flavel, R. A. (1980) *Cell* **19,** 947–958
Varshavsky, A. (1981) *Cell* **25,** 561–572
Varshavsky, A., Bakayev, V. V., Nedospasov, S. A., and Georgiev, G. P. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 457–473
Vidali, G., Boffa, L. C., and Alfrey, V. G. (1977) *Cell* **12,** 409–415
Walker, J. M., Hastings, J. R. B., and Johns, E. W. (1977) *Eur. J. Biochem.* **76,** 461–468
Weintraub, H., and Groudine, M. (1976) *Science* **193,** 848–856
Weisbrod, S., and Weintraub, H. (1981) *Cell* **23,** 391–400
Weisbrod, S., Groudine, M., and Weintraub, H. (1980) *Cell* **19,** 289–301
Wells, J. A., and Yount, R. G. (1980) *Biochemistry* **19,** 1711–1717
Wilkinson, K. O., Urban, M. X., and Haas, A. L. (1980) *J. Biol. Chem.* **255,** 7529–7532
Wu, R. S., Kohn, K. W., and Bonner, W. M. (1981) *J. Biol. Chem.* **256,** 5916–5920

Downloaded from http://www.jbc.org/ at CALIFORNIA INSTITUTE OF TECHNOLOGY on November 25, 2020

On the chromatin structure of the amplified, transcriptionally active gene for dihydrofolate reductase in mouse cells.

J Barsoum, L Levinger and A Varshavsky

J. Biol. Chem. 1982, 257:5274-5282.

Access the most updated version of this article at http://www.jbc.org/content/257/9/5274

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/257/9/5274.full.html#ref-list-1

Downloaded from http://www.jbc.org/ at CALIFORNIA INSTITUTE OF TECHNOLOGY on November 25, 2020